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Research report

Serotonergic activation of locomotor behavior and posture in one-day old rats

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HIGHLIGHTS

- Quipazine induced air-stepping, locomotion, and posture in P1 rats.
- During air-stepping, steps maintained highly anti-phase coordination.
- Forms of locomotion included pivoting, crawling, and some walking.
- Advanced posture such as head elevation and locomotor stances were shown.

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ABSTRACT

The purpose of this study was to determine what dose of quipazine, a serotonergic agonist, facilitates air-stepping and induces postural control and patterns of locomotion in newborn rats. Subjects in both experiments were 1-day-old rat pups. In Experiment 1, pups were restrained and tested for air-stepping in a 35-min test session. Immediately following a 5-min baseline, pups were treated with quipazine (1.0, 3.0, or 10.0 mg/kg) or saline (vehicle control), administered intraperitoneally in a 50 μL injection. Bilateral alternating stepping occurred most frequently following treatment with 10.0 mg/kg quipazine, however the percentage of alternating steps, interlimb phase, and step period were very similar between the 3.0 and 10.0 mg/kg doses. For interlimb phase, the forelimbs and hindlimbs maintained a near perfect anti-phase pattern of coordination, with step period averaging about 1 s. In Experiment 2, pups were treated with 3.0 or 10.0 mg/kg quipazine or saline, and then were placed on a surface (open field, unrestrained). Both doses of quipazine resulted in developmentally advanced postural control and locomotor patterns, including head elevation, postural stances, pivoting, crawling, and a few instances of quadrupedal walking. The 3.0 mg/kg dose of quipazine was the most effective at evoking sustained locomotion. Between the 2 experiments, behavior exhibited by the rat pup varied based on testing environment, emphasizing the role that environment and sensory cues exert over motor behavior. Overall, quipazine administered at a dose of 3.0 mg/kg was highly effective at promoting alternating limb coordination and inducing locomotor activity in both testing environments.

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1. Introduction

Mature locomotor behavior involves the ability to alternately flex and extend the limbs while maintaining postural stability, as well as the ability to traverse various terrains and make changes in movement direction [1]. The mechanisms supporting locomotion begin developing prenatally in most species, but in altricial animals, such as rats and humans, a large portion of this development continues after birth as well. This is largely due to the fact that the ontogeny of locomotion does not involve a single system, but is dependent upon the development of various systems (*i.e.*, sensory, motor, postural, neurotransmitter, etc.). This is exemplified in the case of the rat [2]. Locomotor-like alternation of the forelimbs and hindlimbs is expressed as early as gestational day 20 (2 days before birth) in the rat [3,4]. While some of the basic motor coordination mechanisms for locomotion are present prenatally, the newborn must adjust to the demands of a terrestrial environment, such as exhibiting postural control to counteract gravity, to engage in more complex locomotor behavior. During the prenatal period, the fetal rat does not have gravitational constraints placed on limb movement in utero as it does when attempting locomotion in the postnatal environment [5]. This adjustment to locomotion in a gravitational environment, for an animal with

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relatively weak anti-gravity extensor muscle control [6], may help to explain why neonatal rats and other altricial newborns exhibit little spontaneous locomotion at birth.

Over the first two postnatal weeks, rat pups show limited, but gradual changes in their locomotor behavior. Immediately after birth they maintain a flaccid posture with limbs extended away from the body [7]. Control of the forelimbs and shoulders of the rat begin to mature during the first postnatal week, allowing the animal to support its weight with the front portion of its body. At the end of the first postnatal week, rat pups begin to exhibit pivoting behavior and some crawling. However, it is not until the second week that the hindlimbs begin to catch up to the forelimbs, permitting expression of more mature patterns of locomotion, including more frequent crawling and walking, which gradually become more coordinated and adult-like [7]. Changes in locomotor behavior are accompanied by developmental changes in limb motoneuron response properties [6], muscle activation patterns [8], and descending supraspinal pathways [9].

Given the gradual development of locomotor and postural behavior, researchers have established the air-stepping paradigm to permit the study of early locomotor development *in vivo* [10–12]. The air-stepping paradigm is a useful model for studying the early development of locomotion because it alleviates the gravitational and postural constraints of terrestrial locomotion. This procedure involves suspending the immature animal in a sling, so it can exhibit locomotor limb activity without the necessity of counteracting gravity. Previous studies in our lab, as well as others, have shown that sustained periods of air-stepping (defined as the limbs moving in a locomotor-like pattern while the animal is suspended off the floor) can be induced in the neonatal rat by activating the serotonin or dopamine systems [10–12]. Additionally, air-stepping has been evoked using olfactory stimuli (*i.e.*, bedding material) [13]. When pups are suspended off the ground and presented with bedding material from the nest, they exhibit air-stepping behavior [13]; however, olfactory-induced stepping does not appear to be as sustained as drug-induced stepping.

To evoke air-stepping in rodents with serotonergic stimulation, the serotonin receptor agonist quipazine is often used [4,11,14,15]. The effects of quipazine on stepping are blocked by pre-treatment with a 5-HT_2 antagonist, providing evidence that quipazine acts at 5-HT_2 receptors [16,17]. Quipazine-induced stepping has been used to study the development [4,11], sensory regulation [18,19], and spinal mechanisms [14,16,15,17,19] of locomotor activity. A dose-response curve conducted in fetal rats *in vivo* found that 3.0 mg/kg of quipazine evoked significantly more alternating steps (over 15 times more) compared to saline control subjects [4]. However, since most studies utilize postnatal rat pups in the air-stepping paradigm, especially to study mechanisms regulating stepping behavior, it is imperative to examine this issue in postnatal pups. Furthermore, although quipazine evokes stepping behavior, the effect of quipazine dose on interlimb coordination parameters, such as step period and interlimb phase, has not been investigated. Thus, one purpose of the present study was to conduct a dose-response curve for quipazine in postnatal rat pups, to assess effective dosage, step period and interlimb phase during quipazine-induced stepping. This information is important to know for our understanding of how this air-stepping model relates to coordinated locomotion.

While the air-stepping paradigm provides a model to study early development of locomotor behavior, it is important to point out that air-stepping is not locomotion per se. Locomotion requires not only movement of the limbs, but also postural control and the propulsion of the body through space. Neonatal rats that exhibit air-stepping do not move their entire body through space or across an area, but rather only move their limbs while the body remains stationary (*i.e.*, secured to the bar from which they are suspended). Crawling in the immature rat has been induced with olfactory stimuli, such as nest and bedding odor at postnatal day 0 (P0) [13] and amniotic fluid and milk at P1 [20]. But unlike quipazine-induced stepping, locomotor activity induced by olfactory stimulation is usually very brief in duration. Nonetheless, these experiments demonstrate that the newborn rat has the ability to at least show brief bouts of crawling locomotion.

The duration and types of locomotor and postural patterns (*i.e.*, crawling, walking locomotion, quadrupedal stance) that quipazine evokes in the freely moving neonatal rat is the second main focus of the present study. Spear and Ristine [21] demonstrated that quipazine is capable of evoking increased locomotor behavior in P3 rats, at a dose of 10.0 mg/kg. Compared to that study, here we test younger pups, examine locomotion and posture in a larger testing arena, classify several additional locomotor and postural behaviors, and compare the effect of different quipazine doses on such behaviors. Thus the experiments in this study are aimed at assessing how closely the quipazine-induced stepping paradigm relates to actual locomotion and identification of the most effective dose of quipazine for evoking locomotor behavior in neonatal rats.

2. General methods

2.1. Subjects

Subjects were Sprague-Dawley rats bred in the Animal Care Facility at Idaho State University. Subjects remained housed in the home cage with the dam until testing. Testing occurred on P1 (24 h after birth). Animals were examined prior to testing to ensure that they had fed recently as indicated by the presence of a milk band on the abdomen, and were in overall good health (*e.g.*, pink in color). A total of 56 P1 rat pups were used as subjects in the two experiments. In order to avoid litter effects [22], no more than one pup per litter was assigned to each group. Animal care and use were in accordance with NIH Guidelines [23] and the Idaho State University Animal Care and Use Committee.

2.2. Behavioral testing

On day of testing, pups were individually tested inside an incubator that maintained humidity (~40%) and ambient temperature (at 35 °C). They were manually voided for up to 20 s or until urination/defecation, by gently stroking the perineum with a small paintbrush. Pups were then placed inside the incubator for 30 min prior to testing to allow for acclimation to testing conditions. Following acclimation, pups received an intraperitoneal (IP) injection of quipazine or saline.

2.3. Pharmacology

Quipazine maleate, a serotonergic receptor agonist (Sigma–Aldrich, St. Louis, MO) was prepared in doses of 1.0 mg/kg, 3.0 mg/kg, or 10.0 mg/kg. Doses administered were based on a previous study in fetal rats [4], as well as previous studies with newborn rats that used 3.0 mg/kg quipazine to evoke alternating stepping [10,11,18,19]. Pups in the control group for both experiments received saline (vehicle control). Drugs were administered through a 0.05 mL IP injection with a 30-gauge needle. The researcher was blind to drug condition during administration.

3. Experiment 1: effect of quipazine dose on interlimb coordination and step period

Although quipazine is often used to evoke locomotor-like stepping behavior in newborn rats *in vivo*, step coordination during